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BY JOHN DAY, M.D., M.R.C.S.E.



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## ON COLOUR TESTS AS AIDS TO DIAGNOSIS.

By JOHN DAY, M.D., M.R.C.S.E.

A Paper read before the Medical Society of Victoria, 6th October.

Mr. President and Gentlemen,—I have the honour, this evening, to bring under your notice some new and very delicate tests, by which the presence of blood, pus, mucus, and saliva, may be readily detected, and as, though each test possesses its own distinctive character, they all in their reactions oxidize and change the colour of guaiacum resin, I have called them Colour Tests.

I should, perhaps, here remark that, in speaking of these Colour Tests as aids to diagnosis, I do not limit the meaning of the word to the mere recognition of disease, but use it in its broadest acceptance.

The delicacy and reliability of the test for blood have already been fully recognised by Professor Taylor, in a paper "On the Guaiacum Process for the Detection of Blood in Medico-Legal Cases. The Antozone Test," published in Guy's Hospital Reports, vol. xiii. By the last mail I had the pleasure of receiving a letter from Professor Taylor, in which he makes the following remarks, which I need hardly say are, to me, very gratifying:—"I have nothing new to add about the guaiacum process for blood. It is wonderfully delicate and quite satisfactory when used with caution. I have employed it on a variety of occasions in medico-legal cases. The presence of blood in the contents of the stomach and in articles of food in cases of poisoning, may now be determined with the greatest ease by means of this process. There are many cases in which it can be used where the spectroscopic microscope utterly fails to show blood, and there is no case in which the spectroscope is available for use in which this method by guaiacum is not equally efficacious."

The guaiacum process for the detection of blood is an application of a discovery made by the late Professor Schönbein, who found that peroxide of hydrogen, which, according to his views, is an antozonide and composed of water and antozone, is rapidly decomposed in the presence of blood, its antozone being converted into ozone by mere contact with the corpuscles. In order to prove the correctness of these views, he made use of tincture of guaiacum, the precipitated resin of which is chemically indifferent to antozone and undergoes no change of colour in its presence, but is possessed of a strong affinity for ozone, with which it combines and becomes changed from its normal colour to a bright blue.

About three years since, I attempted to repeat the experiments by which Schönbein arrived at the discovery just alluded to, but found it impossible to procure the peroxide of hydrogen. It is a substance, as described by writers on chemistry, not only very difficult to make, but when made is so prone to decomposition that it cannot be preserved without the aid of hydrochloric acid, and this renders it comparatively valueless as a test for blood in cases where great delicacy is required, oxidation and blueing of guaiacum resin by the action of ozone being checked in the presence of free acids.

It was not long, however, before I was so fortunate as to find that antozonides of a very stable character, and strictly resembling peroxide of hydrogen in all their reactions, were the easiest possible compounds to make, and, moreover, that they exist in abundance, ready made, in every chemist's establishment. This spontaneous formation of peroxide of hydrogen or antozone in medicinal preparations is a subject well worthy of the attention of our leading pharmaceutical chemists; for in undergoing this change they acquire altogether new therapeutic and physiological properties. There are certain drugs which have a special tendency to absorb oxygen in this form and to become antozonised. Among them I may mention ether, oil of turpentine, oil of lavender, oil of juniper, oil of lemon, oil of eucalyptus, oil of amber, and, indeed, I think I may safely say that all the essential oils are capable of undergoing this change under favourable conditions. This process takes place more rapidly in essential oils when they are combined with alcohol. It is not, perhaps, generally known that when these oils become highly charged with antozone they acquire the property of combining in all proportions with alcohol. The bottles on the table marked *A* all contain antozonised fluids which have been artificially prepared. Those marked *B* contain fluids which have become antozonised by a natural process. That they all contain antozone may be readily proved by first adding a drop or two of any one of them to a few drops of tincture of guaiacum, which will produce no change in its colour, antozone having no affinity for guaiacum resin; but on placing the smallest particle of blood in contact with this mixture a beautiful blue tint will be developed. This result, according to Schönbein, is due to the fact that oxygen in the form of antozone cannot exist in the presence of blood, but is instantly transformed into ozone, which, having a greater affinity for the guaiacum resin than it has for the constituents of the blood, combines with it and produces its characteristic blue reaction.

Having now at my command an abundant supply of peroxide of hydrogen or antozone, I began to experiment with it pretty freely, and soon found one sample of compound spirit of sulphuric ether which had become so highly antozonised that it immediately gave a bright blue reaction with guaiacum in the presence of blood, even when the quantity of blood was so minute as to be quite imperceptible to the naked eye. This led me to think of bringing the

subject under the notice of Professor Taylor. There were, however, certain difficulties to be overcome, before this process for the detection of blood could be made available in medico-legal inquiries; the greatest, perhaps, consisting in the large number of substances, both organic and inorganic, which act on guaiacum and turn it blue. This difficulty, however, is removed by the fact that, with one or two known exceptions, which may be easily recognised, they all act on the guaiacum alone; whilst blood produces no change in the colour of guaiacum, except in the presence of antozone. It is clear, therefore, that in testing for blood by this process we must always apply the guaiacum first, and if it be turned blue by the substance under examination, we may infer, either that it is not blood, or, if blood, that there is also some substance present which can blue guaiacum without the intervention of antozone.

The difficulty of recognising the blue reaction on dark cloth at one time threatened to mar the value of this process, but it has been overcome by the simple expedient of placing a piece of white blotting-paper over the cloth immediately after the tests have been applied, and gently pressing it. By this means perfect impressions of every minute spot of blue may be readily obtained.

In November, 1866, I wrote to Professor Taylor, and asked his opinion regarding the value of this test, and I enclosed in my letter a small piece of dirty brown cloth, on which were minute traces of blood. This was kindly given to me by Mr. Johnson, the Government Analytical Chemist, who had previously examined it and failed to recognise blood by means of chemical tests, although by aid of the microscope he had discovered blood corpuscles. By the guaiacum process and blotting-paper I succeeded in striking off from this piece of cloth upwards of sixty impressions, each containing bright blue spots. In the early part of the succeeding year Professor Taylor examined the piece of cloth just referred to, and made the following report:—"The material was a close cotton fustian of a dirty brown colour. I could not by the aid of the microscope detect upon the stuff any stain or spot resembling blood, nor could I extract any red colouring matter from the stuff by cutting it into small fragments, digesting them in water, and afterwards compressing them. A part of the fibre, which was somewhat stiffened, was wetted with tincture of guaiacum, which produced no change of colour in the stuff, and peroxide of hydrogen was afterwards added. The blue colour which is caused by the red colouring matter of blood under these circumstances was not apparent on the brown fustian, but on pressing the wetted spot on white blotting-paper two faint blue impressions were obtained; thus corroborating Dr. Day's results some months after the performance of his experiments."—*Guy's Hospital Reports*, vol. xiii., p. 432.

I will now show you these Colour Tests, and offer a few remarks on their properties, and the purposes to which it has appeared to me they may be applied.



The principles on which the blood-test is based have already been explained. It is so easy of application that I need not occupy much of your time in showing you how it should be used. There is, however, one step in the process which must never be forgotten, and that is, invariably to apply the guaiacum first, and if this be followed by a negative result, then to add a few drops of antozonised ether, when, if blood be present, oxidation and blueing of the guaiacum will speedily follow.

Although, as I have already stated, a large number of substances are capable of becoming antozonised and, as a consequence, of turning guaiacum resin blue in the presence of blood, I would much prefer, for medico-legal investigations, the use of Robbins's Ozonic Ether. It is a very carefully prepared ethereal solution of peroxide of hydrogen of an uniform strength, which is an obvious advantage in cases where great delicacy is required. The tincture of guaiacum used in this test need not be strong. It should be freshly prepared and in a perfectly unoxidized condition. I make it for my own use by putting a few pieces of pure and unoxidized guaiacum resin into a small bottle and filling it up with alcohol ; after shaking it for a minute or two it is fit for use. It should be kept in the dark.

The piece of paper I now show you contains traces of blood upwards of twenty years old : on applying tincture of guaiacum to it no change of colour will occur, but on adding a drop or two of ozonic ether the guaiacum will be rapidly oxidized and acquire a bright blue colour.

The test for pus is made by exposing a saturated alcoholic solution of guaiacum to the air until it has absorbed a sufficient quantity of oxygen to give it the property of turning green when placed in contact with iodide of potassium. Perfectly sound tincture of guaiacum, such as should be used for the blood-test, undergoes no change of colour in the presence of iodide of potassium.

On this piece of paper there is a small quantity of pus. It is more than nine weeks old, and yet you will see that on moistening it with water and pouring a drop or two of the oxidized tincture of guaiacum over it a clear blue colour will be produced. In operating on pus which has become dry, it is always necessary to moisten it with water before applying the test.

Although, for convenience sake, I have spoken of the tincture of guaiacum used in this test as oxidized, I am more disposed to think that in reality the oxygen is held in a state of solution, much as it exists in the blood.

I can offer no very satisfactory explanation of the reactions which give rise to the blueing of oxidized guaiacum by pus. It may be that pus possesses the property of chemically polarizing the neutral oxygen contained in the tincture of guaiacum and splitting it up into ozone and antozone, and that the ozone thus generated oxidizes and blues the guaiacum. I may here remark, that prolonged exposure to air and light causes tincture of guaiacum to absorb

antozone. It will then turn blue in the presence of blood alone, and is unfit for use in any of the colour tests.

The test for mucus consists in the application, first, of oxidized tincture of guaiacum, which by itself undergoes no change in the presence of mucus, and then, in the application of carbolic acid or creasote, which quickly changes the colour of guaiacum to a bright blue, although neither carbolic acid nor creasote will blue guaiacum alone. In testing for mucus on cloths, or when it is mixed with blood, it is necessary to use the carbolic acid pure, but when the mucus is in a liquid state, it is better to use carbolic acid diluted with alcohol.

The cloth I am now about to show you has been nearly saturated with menstrual discharge, and as there is always mucus mixed with this fluid it affords a good opportunity for enabling you to judge of the value of this test. On pouring over it a small quantity of oxidized tincture of guaiacum no change of colour will occur, but on adding a little pure carbolic acid or creasote, a bright blue will be quickly produced. The coloured diagram on the table shows very fairly the blue reaction produced by this test when applied to a napkin used by a person recently confined.

The test for saliva is similar to that for mucus, with the exception that the blue reaction produced by the oxidized tincture of guaiacum and alcoholic solution of carbolic acid is highly intensified by the addition of ozonic ether, or any other antozonised fluid. That this is not due to the presence of blood may be easily proved by placing a small quantity of saliva in a test tube and allowing it to stand for a few days; on carefully taking one drop from the surface and applying the test for saliva, the above named indications may be obtained, but on taking a second drop and applying the blood-test, a negative result will ensue. I may state that saliva reacts on oxidized guaiacum when combined with carbolic acid, just as readily after the ptyaline has been removed.

There is, on this piece of white blotting-paper, some saliva fourteen days old. On applying the tests to it you will presently see that it still retains the power of giving its characteristic reaction.

I will now conclude by mentioning some of the purposes to which these tests may be advantageously applied.

To those engaged in medico-legal investigations the blood-test is of acknowledged value. The tests for pus and mucus add greatly to its usefulness; as they may enable a medical expert to at once decide on the source from whence blood has been derived.

As aids to medical and surgical diagnosis, these tests bid fair to be of great service. The ease with which, by their agency, the presence of minute traces of blood and pus may be recognised in sputum and urine, renders it unnecessary for me to occupy your time in pointing to cases in which they may be usefully applied. I will mention, however, that in surgical cases, where any doubt exists regarding

the presence of pus in deeply seated parts, the use of a grooved needle and the pus test would at once decide the question.

As blood, pus, mucus, and saliva, may each be distinctly recognised by these tests, and it is probable that their reactions depend on the properties of their corpuscles, I venture to hope that these reagents may assist physiologists in determining the correctness of Cohnheim's views regarding the identity of the white corpuscles of the blood with pus corpuscles; and also of views more recently expressed by Dr. Jos. G. Richardson, formerly Resident Physician at the Pennsylvania Hospital, in a paper "On the Identity of the White Corpuscles of the Blood with the Salivary, Pus, and Mucous Corpuscles." Published in the 35th number of the *Quarterly Journal of Microscopical Science*.